INVASIVENESS OF HELMINTH LARVAE*

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THE invasions of tissues and cells by animal parasites are often spectacular processes when compared to the invasions of the host by bacteria. In some instances these invasions demand prolonged tissue migrations before the invading organism reaches the site within the host where it may survive and develop to maturity. With regard to bacterial invasion we have well documented evidence concerning many of the mechanisms involved and a basis for understanding the variations in the ability to invade found in different groups or strains of organisms. These have been related to toxin and enzyme production or to the possession by microorganisms of substances tending to negate host defenses. However, as a result of our lack of information none of the basic texts dealing with parasitism by animal agents includes specific discussion of this obviously fundamental process in successful parasitism of a host. Invasiveness of parasites in the broadest sense has been discussed by Taliaferro (1). He has pointed out that the invasiveness of parasitic organisms is a summation of a heterogeneous collection of factors which include specialized structures and specific biochemical entities produced by the parasite. Relative invasiveness of a parasite must also take into consideration the diverse host factors tending to resist invasion. The term invasiveness also implies that the invader is able to survive and reproduce. The following discussion will be limited to evidence and speculations concerning the mechanisms involved in the direct invasion of tissues by helminth larvae and to those host responses which may be related to these mechanisms.

The invasion of intact epidermal or mucosal membranes of the host followed by extensive migration through the deeper tissues is an essential portion of the life cycle of many helminth parasites. There exists a relative abundance of detail on the course taken by invading larvae during migration and development in both definitive and intermediate hosts. There is also a considerable literature describing histologic and cellular

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changes in the host as a result of or in response to these tissue invasions. However, our knowledge of the precise mechanisms which enable helminth larvae to penetrate tissues and migrate through them is relatively meager. Correlatively our understanding is slight of those hosts responses which are directed specifically against these mechanisms.

In some groups of helminth larvae, as in the stylet cercariae, it is probable that the major mechanism of invasion is mechanical, aided by secretory processes. In others, as in the filariae, the initial site of entry into the host may be by way of a break in the epithelium made by an agent other than the parasite. There are, however, a large number of tissue-penetrating helminths which are ill equipped for penetration of intact tissue barriers by physical means alone, although muscular locomotor activity is an integral part of the process. Some of these such as the *schistosomes, Strongyloides, Ancylostoma,* and their relatives, are readily capable of passing through the epidermis and into the dermis in a matter of a few minutes. Glandular structures that might secrete histolytic or other substances enabling the parasite to penetrate tissues are prominent features in many of these forms. In numerous instances where alteration of host tissues has been observed a causal relationship has been ascribed to the secretions of glands of larvae, adults, or embryonated eggs in the tissue (2-11). Until relatively recently, all of our information on parasite-induced changes in the host occurring during penetration or tissue migration has been based on indirect evidence of this nature. The first exception to this was the observation by Davis in 1936 (12) that a homogenate of a dermatitis-producing cercaria (*Diplostoma*) possessed a proteolytic activity that would cause "pulping" of bits of frog muscle. A similar activity was reported in 1937 by Hunter (13) for the cercariae of *Cryptocotyle.*

More recently a number of investigations have been made of parasite secretions and host-parasite interaction which give us the beginning of a more detailed concept of the nature of the enzymatic invasion mechanisms of helminths and the host response to these processes. These investigations have been more or less directly stimulated by advances in our knowledge of bacterial invasiveness as well as by the development of methods and advances made in histochemistry, microchemistry and immunology.

The first of these enzymatic mechanisms of invasion to be discussed is one in which the activity of certain larvae is to a great extent directed against the acellular constituents and intercellular cement substances of the tissues. During their brief and often rapid passage through the skin, helminth larvae must traverse the non-living stratum corneum, the cellular layers of the epidermis, and then the acellular barriers of the basement membrane and ground substance of the dermis. These acellular areas

have as their major constituent polysaccharide-containing protein which for convenience can be termed glycoprotein. If it is necessary for the parasite to enter the circulatory system or pass through other epithelia of the host it again encounters similar glycoprotein barriers surrounding the vessels or underneath the epithelium. The nature and characteristics of these acellular substances have been elucidated through a number of histochemical studies which show that in the mammalian host the degree of polymerization or aggregation of the polysaccharide-containing protein can be correlated with the intensity of staining by the Hotchkiss (Periodic Acid-Schiff) and Evans blue techniques (14, 15, 16). The basement membrane of the skin for example, in the adult host, is a thin, dense, highly polymerized, continuous sheet extending beneath the stratum germinativum. It forms a homogeneous matrix in which various fibrous elements may be embedded and it appears to have continuity with intercellular cement substances as well as with the less dense ground substance. Changes in the density of these glycoprotein elements with age or parturition and in disease processes are detected histochemically as a decrease or increase in staining intensity or loss of material with the Hotchkiss method or an increased or decreased binding of intravenously introduced Evans blue (14-17). Decreased density and loss of staining ability with the Hotchkiss method and increased binding of Evans blue is interpreted as indicating that the glycoprotein substances have depolymerized and that there is an increase in water-soluble components. A change of this type may be effected by collagenase enzymes. We have shown (18) that during skin penetration by *Schistosoma mansoni, Schistosomatium douthitti,* or *Strongyloides ratti,* alterations of the basement membrane and ground substance occur as shown by the Hotchkiss technique. They appear to soften or depolymerize. The basement membrane disappears prior to the actual entrance of the cercariae into the dermis and the ground substance becomes pale about the cercariae as they enter the dermis. In addition, as these larvae penetrate the skin, Evans blue is bound at the site of penetration as the acellular basement membranes and ground substance become more soluble. Similar but less extensive alteration of glycoprotein occurs about the eggs of schistosomes moving through tissues and at the site of penetration of the snail host by schistosome miracidia. Another parasite affecting acellular glycoprotein of tissue in the same manner is the hexacanth embryo of *Taenia saginata.* As it penetrates the intestinal mucosa (19) secretions of the larva alter intercellular cementing substances and also have some cytolytic effects. A further example of this type of activity is found in the developing larvae of *Taenia taeniaeformis* (20). During the first few days of its development in the liver of the rat, a period which is characterized by rapid growth

of the organism and displacement of hepatic cells, the developing strobilocercus causes marked changes indicative of enzymatic softening of the basement membranes and intercellular substances in its vicinity (20).

Other effects of tissue penetration that are to be noted in those forms that have demonstrable effects on host glycoprotein are changes in collagen and reticulin. Reticular fibers may be seen on occasion to be broken or to have lost their argyrophilia, and collagen fibers may become argyrophilic in connective tissue through which larvae are passing. In addition to evidence of enzymatic alteration of host glycoprotein other changes may be observed. After massive exposure of the skin to larvae of S. *mansoni* or S. *ratti* there is a decrease in protein-bound sulfhydryl groups of the dermis (21). This has been interpreted as a non-specific effect as it is probable that closely following the considerable alteration of material in this area to a more soluble state there is removal of the soluble products by the host circulation. Another effect not entirely related to the effect on glycoprotein is an increase in free water and an increased metachromosia about the penetration site. The latter two phenomena are common features in skin invasion by schistosomes and *Strongyloides*, both of which extensively alter the basement membrane and ground substance. However, they are also found following skin penetration by *Nippostrongylus muris* and tissue penetration by *Trichinella* neither of which exhibit histochemical evidence of enzymatic activity against the polysaccharide-containing protein. As mentioned previously, with peneration of the tissues there is usually a certain amount of cell destruction. However, in our experience and in the experience of others (4, 18, 19) cytolysis has not been an immediate prominent feature accompanying penetration of the tissues by individual helminths. The usual picture is one in which only one or two cells of the epithelium in the immediate vicinity of a larva are lysed; or a few cells may be freed and displaced.

It should be pointed out that in a number of those parasites which cause alteration of glycoprotein during penetration, e.g., the schistosomes and cestodes, the glands actively secreting at this time have as a major constituent a polysaccharide-protein substance. Prior to being secreted this is granular in appearance and when secreted may be deposited in strings or strands on the surface of the host epidermis or may be carried along with the pentrating larvae. In some instances secretions from glands of a similar nature in cercariae may coat the outer portions of the organism. It has been suggested that the secretion is an enzyme-polysaccharide complex (18-19) that by its viscous nature tends to localize the site of activity of the enzyme (18) or that it is a mucoid substance protecting the invading organism from the defense mechanisms of the host (23, 24).

The living eggs of schistosomes in tissues similarly have a glycoprotein secretion closely applied to their external surface (18, 22) and the miracidial penetration glands have a similar constituent. The presence of secreted polysaccharide about these organisms is extremely interesting when compared to the situation found in some invasive bacteria. In these the capsules are polysaccharide in nature and may contain some amino acid. The capsules function to protect the organism by inhibiting phagocytosis or digestion by host cells, and the introduction of capsular material into the host aids invasion by ordinarily noninvasive unencapsulated organisms. It is possible that the secretions play a dual role in serving to localize a readily diffusible enzyme and to protect the parasite from the host. It would be interesting to determine whether these polysaccharide substances are related to those shown by Oliver-Gonzalez (25) to be important antigen components in these infections.

The evidence of enzymatic activity of helminth larvae derived from histochemical studies is not precise since it does not elicit information on the exact nature of the enzymes altering the tissue components of the living host. However, the effects seen during these helminth invasions are remarkably similar to those resulting from the activity of certain bacterial toxins, particularly the collagenase-containing toxin of *Clostridium welchii A* (14, 16). Similar enzymatic activities and histochemical findings have been described in rapidly metatasizing tumors in locales where they are invading normal tissue (14, 16). More direct evidence that this similarity is more than superficial is available in the case of the cercariae of S. *mansoni* and S. *douthitti,* for the filariform larvae of S. *ratti* and for the early stages of development of the larvae of *T. taeniaeformis* (18). Most information exists for S. *mansoni* and S. *ratti,* and the following pertains primarily to these forms. Secretions of the living larvae are, like bacterial collagenase, capable of digesting thin gelatin films, and releasing dye from azo-dye-bound collagen substrates. Saline extracts of the larval homogenates are similarly active against these substrates, exhibit a general proteolytic activity against frozen dried tissue sections and also show extensive activity against the polysaccharide-containing protein of such sections. The activity of these saline extracts of helminth larvae in releasing dye from azotized hide-powder collagen and from azotized cartilage has provided a means of quantitative photometric comparison of the activities of different helminth extracts and also allows comparison to the activities of bacterial collagenase and proteolytic enzymes such as crystalline trypsin (21). From these tests it has been shown that the activity of the helminth-derived enzymes is directly proportional to the number of organisms in the test system or to the dry weight of organisms from which the aliquot of enzymes has been extracted. The activity

increases with time when incubated at 37°C and is destroyed by heating at 60°C. In contrast to the collagenase of *Cl. welchii* toxin, which is almost uniformly active over a relatively wide pH range, the enzyme of *S. mansoni* cercariae has a distinct optimum at pH 7.5 while that of *S. ratti* is in the vicinity of 7.0. The enzymes of the helminths and bacterial collagenase differ slightly in the degree of sensitivity to various metal ions. However, all were inhibited by mercury and copper and slightly activated by the presence of calcium and magnesium. Agents binding sulfhydryl groups inhibit the activity of the enzyme derived from *S. mansoni* but have little effect on that of *S. ratti.* The latter is more sensitive to chelating agents and sulfhydryl-containing compounds than is the cercarial enzyme. Both of these substances (S-H-binding and chelating) inhibit the activity of bacterial collagenase. In contrast to trypsin, and like the bacterial collagenase, both helminths' enzymes are active on cartilage and are not affected by the presence of the specific trypsin inhibitors. In comparison to bacterial collagenase, the activity of the helminth enzymes on native collagen of bovine origin *in vitro* is slight with only small amounts of hydroxyproline being released as amino acid or in polypeptides, although *in vivo* activity against collagen-containing tissues of the normal host is marked. The enzyme of *S. mansoni* cercariae, like trypsin, is capable of degrading urea-denatured hemoglobin to release tryosine, an activity which in the case of the helminth extract is not abolished by trypsin inhibitors. The substances inhibiting the helminth enzymes *in vitro,* when present at low concentrations, will in some instances retard or inhibit penetration. It is apparent from these studies that although there are points of resemblance, the enzymes derived from infective larvae of *S. mansoni* and *S. ratti* differ not only from each other but also from bacterial collagenase and trypsin. It should be emphasized again that this type of enzymatic activity is limited to those helminths which histochemically are shown to alter the polysaccharide-containing protein of the host and that this activity is absent in such effective tissue invaders as *Nippostrongylus muris* or *Trichinella spiralis.* Similarly these activities have not been found in extracts of various xiphidiocercariae or pharyngeate strigeid cercariae and only equivocal activity is found in extracts of filariform larvae of *Ancylostoma caninum.*

The parasite species possessing collagenase-like pentration enzymes do not exhibit this activity at all stages in their life histories. Thus, although proteolytic activity can be demonstrated in extract of adult schistosomes, this does not have the characteristics of the enzyme of the cercariae and is not inhibited by the same substances. Histochemical evidence suggests that the embryonated eggs and miracidia of the schistosomes secrete enzymes similar to those used by the cercariae during penetration (18).

Also, the possession of a collagenase-like activity is limited in *T. taeniaeformis* to the early, rapidly growing stage of the strobilocercus and is not found in older stages or in the adult tapeworm (20).

There is no direct information available on the presence of enzymes in other helminths that migrate extensively through connective tissue, but on a purely speculative basis it seems probable that collagenases or collagenase-like enzymes may be present in adult or developing-tissue stages of *Dracunculus, Loa, Onchocerca* and similar forms. Collagenases have, however, been reported from the highly invasive arthropod larvae, *Hypoderma bovis* (26), from the Australian sheep blowfly *Lucilia cuprina* (27), and from *Lucilia sericata* (28).

It has been convenient in the discussion so far to view the activities of those organisms which alter polysaccharide-containing protein during invasion as resulting from a single proteolytic enzyme. However, this is in all probability an oversimplification since the helminth penetration mechanism more probably is a complex system similar in this respect to bacterial toxins such as the toxin of *Cl. welchii* (29, 30). The most obviously useful functional enzyme to be sought for is a mucopolysaccharidase such as hyaluronidase. The discovery of hyaluronidase and its relationship to the invasiveness of bacteria has stimulated a number of investigators to search for a similar activity in animal parasites. A "spreading factor" has been described from larvae of *Ancylostoma duodenale* (31, 32), *Cercaria ocellata* and *Cercaria pseudarmata* (33) and cercariae of *Schistosoma mansoni* (34). In studies from our laboratory we have also described an effect somewhat similar to the "spreading" of hyaluronidase obtained following the intradermal injection of extracts of larvae of *S. mansoni, S. ratti, S. simiae, A. caninum,* and *N. muris,* as well as following the injection of extracts of non-invasive *Rhabditis pellio* (18). This response differs from that produced by hyaluronidase in that the "spreading" is usually accompanied by a swelling of the dermis and the production of a mucoid-appearing bleb that may be seen if the skin of the animal is viewed from the dermal surface. There was no correlation with this "spreading" and the effect of penetrating larvae on polysaccharide-containing protein of the skin.

Another indirect but sensitive method of demonstrating an activity of hyaluronidase, of undetermined specificity, is the streptococcal decapsulation test. This has been applied to the investigation of several parasites and a decapsulating factor has been reported for cercariae of *S. mansoni* (35, 36), and its presence suggested in *A. caninum* and *Entamoeba histolytica* (37). It has also been reported in *Balantidium coli* (38). In our own investigations (39), extracts of cercariae of *S. mansoni,* and filariform larvae of *S. ratti, A. caninum* and *N. muris* were found to be capable of

reducing the size of capsules of streptococci. The extracts of *S. mansoni* cercariae exhibited the greatest activity of those tested. None of the extracts in our preparations were capable of as complete a decapsulation of the bacteria as the hyaluronidase controls.

Our knowledge of the activity of enzymes of skin-penetrating helminths on more precisely characterized mucopolysaccharide substrates under well-controlled conditions is meager. A reduction in viscosity of hyaluronic acid incubated with cercariae of S. *mansoni* has been reported in one investigation in which the activity did not correlate directly with the number of cercariae (40).

Other investigators have been unable to demonstrate hyaluronidase in *S. mansoni* by similar methods (4). We have also been unable to demonstrate hyaluronidase activity in extracts of larvae of *N. muris, S. mansoni, S. ratti, S. simiae,* or *R. pellio* using a highly purified hyaluronic acid substrate derived from the streptococci whose capsules are reduced by these same extracts. In addition none of the helminth extracts tested exhibited activity toward chondroitin sulfuric acid or ovomucin. However, extracts of *S. mansoni* cercariae were found to be extremely active on one mucopolysaccharide substrate, heparin. It is further interesting in this connection that the toxin of *Cl. welchii* also is active against heparin but in addition has demonstrable activity against hyaluronic acid and ovomucin but not against chondroitin sulfuric acid.

The apparent disparity in the results of studies on the presence of mucopolysaccharidase activity in helminths may be due to a variety of factors. The methods that have been used vary greatly in their sensitivity. In addition, as with the "spreading factor" test, the methods used are not necessarily an index of a specific enzyme activity; for example, in some bacterial substances of high "spreading" potency no hyaluronidase is demonstrable (41). In many of the investigations the substrates utilized have been mixtures and the apparent activity measured may well be related to the degradation of substrate components other than hyaluronic acid. No functional significance can be ascribed at present to heparinase activity. The relatively high level of activity per organism in the case of *S. mansoni* suggests the possibility that if a more natural mucopolysaccharide substrate were used a significant level of activity might be found to which a functional interpretation might be ascribed. It is my feeling that at the present stage of our knowledge we can admit the presence of a mucopolysaccharidase activity but that the presence of hyaluronidase in the specific sense of the term is questionable. *In vivo,* hyaluronidase inhibitors do not affect the penetrating capacities of the parasites (21). The finding that mucopolysaccharidase activity is present at a low level in a variety of tissues and undifferentiated epithelia suggests the possi-

bility that the low levels that may be present in these helminth extracts is related to a function other than penetration.

The only remaining enzymatic activity that has been definitely identified in helminth larvae for which a function in invasion has been suggested is the lipase of *Nippostrongylus* described by Thorson (42). He has demonstrated that secretions and extracts of infective larvae are capable of producing fatty acids from a vegetable fat substrate. We have recently demonstrated a lipase capable of acting in a similar manner on tripalmitin, a normal skin constituent, in extracts of filariform larvae of *Nippostrongylus muris* and *Strongyloides ratti,* and in extracts of cercariae of *Schistosoma mansoni* (43). At present the significance of these findings is not clear and we hesitate to place a functional interpretation on their presence.

Up to this point of my discussion we have been concerned with the observed or measured effects of the parasite on certain of the normal host substrates that are encountered during initial invasion or passage through tissues. During this process the parasite is exposed to a multitude of factors and host substances any one of which may alter the ultimate success of the invading individual. One of these we have not previously mentioned is the state of the stratum corneum. The relative success of the invading individual has been shown by Stirewalt (44) to be influenced by its integrity and the nature of the surface. I should like to emphasize that, excluding the other factors, the physical or physiological state of one of these substrates, the host glycoprotein, may be of great significance to the relative success of the invading parasite. It is a barrier to penetration that fluctuates in its effectiveness and in its physical integrity with the age or physiological state of the host (14, 17). The basement membranes and ground substance of young animals are diffuse and nonresistant, allowing skin-penetrating helminths to traverse them with ease and rapidity. In older animals these are thicker and denser and more resistant to enzymatic alteration. Consequently not only is penetration slower in older animals but also a relatively high percentage of the larvae are retained in the skin, excluded from the dermis by the unaltered basement membrane. Hypophysectomized young rats develop highly polymerized basement membranes in the skin and resemble aged animals in this respect. When speed and relative success of penetration of these are compared with those of normal litter mates the hypophysectomized animals exhibit a condition analogous to that of the older animals in that larvae take longer to penetrate and many are not readily able to alter the basement membrane and gain access to the dermis. This suggests that the age immunity operative in some parasitic infections has non-humoral aspects and may develop in the absence of natural antibody or an antibody

response engendered by previous exposure to homologous or related antigens. It also suggests the possibility that the absolute or relative immunity of a species to particular skin-penetrating parasites might be related to the constitution of this glycoprotein barrier. It is possible that slight species differences in the chemical nature of the barrier would make it partially or completely resistant to the parasite enzyme system. This concept might explain the usually superficial nature of cutaneous larval migrans as discussed by Beaver (45) and the observations that traumatic damage to the skin is in some instances followed by penetration and deeper migration of canine hookworm in man (46, 47). It is interesting in this respect that the larvae of *Ancylostoma caninum* have realtively weak enzymatic activity against glycoprotein when compared to that of the schistosomes and *Strongyloides* (21). These cases of age and species immunity might then be considered a special type of athreptic immunity, as they would be dependent on the presence or state of substrate that cannot be satisfactorily altered by the parasite enzymes.

Certain other conditions of the host such as male castration, cryptorchidism, or adrenalectomy also increase the density of the basement membranes (14, 16) and might also increase the resistance of the host to invasion. Although there is little experimental evidence utilizing this type of host in parasitic infections it has been shown that castrated males' resistance to infection by *Taenia taeniaeformis* is increased (48).

Conversely, it is interesting to speculate, even in the absence of completely sufficient experimental evidence, on what may happen if the glycoprotein barriers of the host are changed by normal physiologic or pathologic processes which alter glycoprotein elements of connective tissue in the direction of decreased density and resistance.

In certain normal and pathological conditions, the basement membranes and ground substance become more plastic and less highly polymerized and might presumably more readily be invaded by parasites. Scorbutic animals, for example, have notably depleted, spongy, altered glycoprotein and are known to be more susceptible to invasions including those of pathogenic amebae (49). However, we are without experimental information as to their reaction to parasitic skin-penetrating helminths. Similarly, the lowered resistance of pregnant hosts to helminth invasions reported by various investigators (50, 51, 52) may possibly be correlated with the greater plasticity and lessened resistance of host glycoprotein substances that develops during this time.

This avenue of speculation also allows us to present a rational hypothesis in explanation of some of the perplexing results obtained in parasitic infections of animals treated with various gonadal hormones. For example, it has been shown by Sadun (53, 54) and by Ackert and Dewhirst (55)

that when moderate doses of testosterone, alphaestradiol or diethylstilbestrol are administered to immature male or female chickens, there is an increase in the resistance to infection by *Ascaridia*. This may possibly be ascribed to the earlier maturation of the connective tissue barriers such as the basement membranes which occurs under the influence of the moderate hormone dosage. On the other hand, Sadun (53), has also shown that when heavy doses of testosterone are administered there is a marked decrease in resistance of the host, with approximately four times as many individuals successfully completing the invasion of the host, This may also be related to the state of the sub-epithelial glycoprotein, as following large doses of testosterone or estradiol there is a striking decrease in this natural barrier.

In each of the instances of hormonal influence on invasiveness that have been cited it is obvious that there are other profound physiological changes in the host in addition to the decreased or increased resistance of the connective tissue glycoprotein. For example, important factors also bearing on invasiveness are the direct effect of gonadal hormones on growth and maturation of parasites, and alteration of antibody response. It is possible that since massive doses of cortisone reduce the glycoprotein barrier (14, 16, 56) this is closely related to the enhancement of parasitism by certain helminths (57, 58) following its administration. However, in this instance we must include in our explanation of its effects the important simultaneous inhibition of inflammatory response and the involution of lymphoid tissue and its effects on antibody production.

The final aspect of invasiveness of the host by helminth larvae that I should like to present deals with one limited aspect of the humoral responses of the host to tissue penetration. It is general knowledge that the immune host is able to inhibit the passage of larvae through its tissues, immobilizing the parasites as they pass through the skin or lungs, precipitating their secretions and metabolic products, and ultimately encapsulating and destroying many of the invading individuals. These phenomena have been the subject of many papers and reviews. It has been suggested that specific anti-enzyme antibodies are produced by the immune host; these would include antibodies acting against the penetration enzymes of helminth larvae (59, 60). The direct demonstration of this is not well documented in the case of invading helminth larvae in actively immunized animals. Thorson has shown that the lipase of *Nippostrongylus muris* is inhibited by serum of animals immunized by previous infection and not by normal serums (42). He has further shown that the proteolytic enzymes of *Ancylostoma* are antigenic and that antiserums may be produced that inhibit the enzyme and suppress the infection (61). The effects of various serums on the collagenase-like penetration enzymes

of *S. mansoni* cercariae and of *Strongyloides ratti* larvae have also been studied and compared to the effects on the collagenase-containing *Cl. welchii* toxin and to trypsin (62). Normal serums from a variety of animals at 1/1000 dilution will completely inhibit trypsin even after being heated to 56°C for 20 minutes. A comparable amount of bacterial toxin (calculated as dye released from azocoll) is either unaffected or activated by this treatment. From 50 to 80% of the activity of the equivalent amount of enzyme of *S. mansoni* is lost in the presence of unheated normal serums of various sources at this dilution. Normal human serum heated at 56°C for 20 minutes does not inhibit the cercarial enzyme. However, in active human infection with *S. mansoni* in a series of 55 individuals at 1/1000 dilution of heat-treated serum, there was an average inhibition of enzyme activity of approximately 40%, with serum of some individuals reducing the activity as much as 70%. It was impossible to correlate the possession of a high level of cercarial enzyme inhibitor in infected individuals with age, sex, therapy received, or clinical status of the infection except that the inhibitor was present in individuals with presently or recently active infections. The 10 individuals of the group showing the most strongly positive skin reactions to cercarial antigen exhibited no greater inhibition to cercarial enzyme than did the whole group. In order to determine whether the anti-collagenase activity of serum from infected individuals was related to the serum's other activities against schistosome antigens, selected serums were separated by starch electrophoresis. The various fractions were tested for their ability to agglutinate cercariae, to agglutinate miracidia, to cause circumoval precipitates, for their complement fixing activity, and for their ability to inhibit trypsin (63). It was determined that the inhibitor of the cercarial enzyme migrates in the region of the alpha globulin and is more sharply limited in its distribution than is the serum trypsin inhibitor. The complement fixing antibodies were found to parallel the distribution of the gamma 2 globulins; the cercarial agglutinin is also within the gamma globulin area and rather sharply limited in its distribution. The miracidial agglutinin, although more widely distributed than the circumoval precipitin, has its peak activity in the same fractions of the gamma 1 globulins. Of the several serums tested following starch electrophoresis, all of those having high levels of anti-collagenase activity have also exhibited circumoval precipitins and miracidial agglutinins. Some of these were lacking in perceptible complement-fixing antibody or cercarial agglutinin although a few, including normal serums, had a factor immobilizing or killing cercariae, that migrated even more rapidly than the collagenase inhibitor or albumins. The enzyme inhibitor is storage labile but not nearly so labile as the cercaricidal substances of normal serum described by Culbertson (61). No precipitates are formed when the inhibitor is incubated with cercarial

extracts. Although it gradually disappears from frozen serum over a period of several months it is stable for months after separation from serum by starch electrophoresis. Its activity is lost if the serum is treated with protein precipitants.

Although increased levels of the collagenase inhibitor are found in serums from infected individuals there is no assurance that this is an antibody phenomenon. In case of normal serums from man, mouse and the rabbit, heating of the serum abolishes the inhibition, whereas in those from the monkey, guinea pig and rat the antienzymatic activity is lowered but not abolished. Rats immunized by repeated exposure to *S. ratti* do not have demonstrably higher titers of collagenase-inhibiting substances than do normal animals. Nor do immunized monkeys or mice with active infections have titers against the collagenase of *S. mansoni* that are demonstrably higher than in the normal animal. Antibody has in at least one instance been shown to migrate with the alpha globulin fraction of serum following immunization with antigens derived from the tubercle bacillus (65). In addition this serum fraction may contain C-reactive protein, various glycoproteins including orosomucoid, and inhibitors of hyaluronidase, trypsin, plasmin and hemagglutinin of influenza virus (66, 67, 68). These may increase during infection or following tissue destruction. It has been determined that the inhibitor of the collagenase of *S. mansoni* cercariae is not identical to any of these substances and is not increased in various bacterial infections, rheumatic fever or acromegaly.

In summary, our knowledge and understanding of the precise mechanisms utilized by helminths in penetration of the tissues of the host is very incomplete. Some forms, e.g., *Schistosoma, Strongyloides,* have been shown to possess enzymes that are proteolytic and collagenase-like in nature. These species cause extensive enzymatic alteration of the acellular, glycoprotein, connective tissue barriers of the host as they penetrate. In addition, the same species may possess mucopolysaccharidases and lipases. Other effective tissue-penetrating larvae such as *Nippostrongylus* are lacking in collagenase-like enzymes and are not known to possess mucopolysaccharidase, but do have lipolytic substances. The penetration enzymes are not present at all stages of growth and development of the parasite but in certain forms at least are limited to those stages which actively penetrate or displace host tissues.

From these investigations it is apparent that in addition to the better known humoral and cellular immune responses, another element in resistance of the host to invasion must be considered. This is the state of organization of the acellular glycoprotein that forms the intercellular cement, subepithelial basement membranes, and ground substance of the host. These substances have been shown to vary in their density and

resistance to enzymatic alteration with the age of the host, nutritional status and hormonal influences. These variations correlate in many instance with the relative susceptibility or resistance to invasion that the host displays. It is suggested that this is a major factor in accounting for the increased resistance with age of the host, and the decreased or increased resistance exhibited by hosts under normal or induced gonadal-hormone changes. Species immunity as well may in part be related to the state of these glycoprotein substances, as it is conceivable that species differences in composition might make these more effective barriers with respect to the enzymes of a particular parasite.

Although there is an abundance of evidence that parasite secretions and products cause the production of precipitating antibodies, specific evidence that humoral responses are directed against the parasite penetration enzymes is meager. *Nippostrongylus* lipase is inhibited by serum of animals actively immunized with this species; but the role of lipase in penetration is speculative. The anticollagenase titer is markedly increased in some human infections with schistosomes. Since a lower titer of similar substance is normally present and since the antienzyme is associated with the alpha globulin serum fraction it is at present inappropriate to consider it an antibody. It varies in its amount and effectiveness from species to species and may function in resistance, possibly playing a role in species immunity. In human infection it is possible that, as suggested by Newsome (69), the maturation of the infection may cause release from the host of some non-antibody substance, such as this, which is inimical to developing or migrating worms.

In conclusion, I should like to emphasize that our knowledge of the mechanisms involved in the penetration of host tissues and cells by animal parasites is still relatively superficial, and that our knowledge of the natural or acquired defenses of the host as related to these mechanisms is similarly deficient. It is to be hoped that future investigations will result in the isolation and precise characterization of the enzyme-substrate systems involved not only in helminth invasions but also in the cellular and tissue invasions of protozoa. The information resulting from such studies should amplify our knowledge of the specific immune responses of the host and should also result in a rational concept of the nature of natural immunity and resistance and the reasons for their variability.

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